

APPLICATION OF ACTINOMYCETES IN NUTRIENTS

MANAGEMENT OF GUAVA CROP

POOVARASAN. S¹, SUKHADA MOHANDAS², SITA. T³,
USHARANI. T. R⁴ & PANEERSELVAM. P⁵

^{1,2,4,5}Indian Institute of Horticultural Research, Bangalore, Karnataka, India

³St.Martins college of Engineering, Hyderabad, Telangana, India

ABSTRACT

Actinomycetes are well known for their role in plant growth enhancement and disease management in wide array of crops. Ten actinomycetes isolated from AM fungal spores colonizing guava rhizosphere were studied for growth improvement of guava cv Arka Kiran under polyhouse condition, enhancement of nutrient uptake and their antagonistic potential against Fusarium solani in in vitro. Out of ten isolates the better antifungal activity was observed in Streptomyces cinnamonensis followed by S. avermitilis and S. netropis. Chitinase genes responsible for the antifungal activity were mined from S. cinnamonensis and other isolates and expressed in M15 E.coli cells. The protein bioassay studies showed that out of three isolates the expressed protein samples respective to S. cinnamonensis showed maximum growth inhibition (1.3 ± 0.16 cm) on F. solani followed by S. avermitilis and S. netropis. Increased plant dry matter was observed in S. albidoflavus (0.856 ± 0.0 g) inoculated plants followed by S. violarius (0.437 ± 0.0) along with increased shoot length (15.5 ± 1.63 cm), (15.2 ± 2.4 cm) over control plants. The increased level of N, P and K was observed in treatments containing S. canus (1.93 ± 0.10 %), S. cinnamonensis (0.57 ± 0.01 %) and S. fradiae (6.15 ± 0.12 %). Soil N was found more in S. scabiei inoculated soil (894.24 ± 0.81) and P, K content increased in S. cinnamonensis (44.43 ± 0.84 ppm) (1606.25 ± 0.04 ppm) treatment over control. Among macro nutrients Ca, was found more in S. avermitilis inoculated plants (130.07 ± 4.9 ppm) followed by S. fradiae inoculated soil (2363.6 ± 2.57). Mg content was higher in S. violarius inoculated plants (2.2 ± 0.16 %) and soil (366.16 ± 1.32 ppm). There was significant improvement in micro nutrient (Fe, Mn, Zn) content in plant and soil.

KEYWORDS: Glomus mosseae, Actinomycetes, Nutrient Mobilization. Chitinase, Fusarium solani

Received: Dec 04, 2015; **Accepted:** Dec 12, 2015; **Published:** Dec 18, 2015; **Paper Id.:** IJASRFEB201604

INTRODUCTION

Guava (*Psidium guajava* L.) is one of the important tropical subtropical fruit crops, well known for their rich nutrient content and unique flavour. Guava fruit acts as moderate source for most of the essential vitamins (Samson 1986). It is being cultivated in all most all the parts of India because of wide adaptability to the soil (Gupta et al., 2010). But in recent years the cultivation is threatened by so many diseases and pests. Wilt caused by *Fusarium* sp is the major issue in guava cultivation in India which depletes the entire production severely. From the time *Fusarium* wilt was reported in India (Naz et al. 2013) many attempts have been made to control the disease. The existing control measures like, using synthetic fungicides which have lots of drawback in terms of environmental safety, soil fertility and loss of fruit quality (Mishra and pandey 1999). The biological control of plant disease has facilitated the safest and sustainable crop protection and production. The discovery of beneficial

rhizosphere bacteria and their effective application to soil resulting in growth improvement and biocontrol have found more alternative for chemical fungicides and fertilizers (Bohra et al., 2006; Naz et al., 2013; Sukhada et al. 2013). The effectiveness of various bio-agents in controlling dreaded pathogens affecting major fruit crops have been reported by many workers (You et al., 1996; Diwedi and Shukla, 2002; Lee et al., 2005; Sukhada et al., 2013). There are many microbial components identified from the microorganism which possess high plant growth promotion and broad spectrum antimicrobial activity. Among them the *Streptomyces* sp are well known for their antagonistic effect against different plant pathogens (Srividya et al. (2012) and solubilization of soil bound nutrition (Vyas and Gulati, (2009) and production of phyto hormones (Kaunat, 1969), organic acids and hydrolytic enzymes facilitating plant growth. In the present study the genes responsible for antifungal activity against *F. solani* were cloned and expressed in E.coli and nutrient solubilization, mobilization capacity of 10 different actinomycetes isolates in growth improvement of guava seedlings under poly house condition was elucidated.

MATERIALS AND METHODS

Isolation of Actinomycetes from the AM Fungal Spores

The dominant AM fungal spores (*Glomus mosseae*) were isolated from the soil by using wet sieving and decantation method (Gerdemann and Nicolson, 1963). The isolated spore were surface sterilized and subjected for the molecular confirmation. The identified spores were crushed manually in a sterile eppendroff tube with micro pestle using phosphate buffer and the crude extract were plated on the nutrient agar to isolate its associated bacteria. After incubation period the developed bacterial colonies were separated morphologically and purified on specific media and their identity was confirmed by molecular means as described by us earlier by using RNA polymerase β subunit primer (Sukhada et al. 2013).

Antifungal Activity Against *Fusarium solani*

Pathogen was isolated from the infected plant root and the identity was confirmed with transcription elongation factor (Arif et al. 2012). The identified culture was purified on PDA agar plates for further assay. The antifungal activity of the actinomycetes was tested against the *Fusarium* by using dual culture assay method (Debananda et al. 2009). The isolates which had shown higher growth inhibition against *Fusarium solani* was further subjected for the isolation chitinase gene.

Identification of Chitinase Gene from the Actinomycetes and Protein Bioassay on *Fusarium solani*

The purified g-DNA from the actinomycetes was analyzed with chitinase gene specific primers (F-atgagtcggcaaggagc; R-tcaggttcggtgaaactc). The amplified fragments from the g-DNA were cloned and sequenced for the nucleotide homology and further the amplified fragments were cloned again into the expression vector (PQE 30 UA – Qiagen Germany) and the expression was induced at the concentration of 100 μ g/ml of IPTG for 3-4 hours in M15 E.coli system. After induction period the expressed protein was harvested from the cells by lysozyme enzyme treatment at 37°C for 1 hr followed by ultra sonication. The extracted proteins were further purified through nickel NTA columns (Qiagen-Germany) and stored at 4°C for further assay. The purified proteins were quantified through dye binding method (Bradford 1976) and tested against the fungi by Agar disc diffusion method (Bauer et al. 1996). The zone of growth inhibition was measured after 24 hrs from the time of inoculation.

Growth Enhancement Activity by Actinomycetes

Seven day-old actinomycetes cultures were tested for their growth promotion activity on guava (*P. guajava* L.) cv Arka Kiran. The culture was grown individually in starch casein media at 30°C at 60 rpm until exponential growth phase was attained. Twenty-five ml of each isolate was mixed in 500 g sterile soil and filled in each polybag at 10⁻⁸ cfu g⁻¹ soil. Arka Kiran seeds were surface sterilized using 0.1% HgCl₂ and were sown in each polybag individually. Control seeds were sown in sterile soil without any culture addition. Each treatment had ten seedlings was replicated five time. The bags were kept in a poly house at temperature 31°C, 70% humidity and a day light ranging from 728 to 913 mol m⁻² S⁻¹ and irrigated with tap water. At the end of 5 month, the seedlings were uprooted and their physical parameter like plant height, leaf area and dry weight of shoots and roots were recorded. The experiment was repeated twice and the average of two readings is presented.

Analysis of Nutrient Content in the Inoculated Soil and Plant

Inoculated soil samples were air dried and sieved properly. Sieved samples, 0.5g each were taken for the analysis of organic carbon content by using Walkley and Black's method (1934). The total phosphorous was estimated by using Bray and Kurtz (1945) method and the other nutrients like potassium, calcium, Zinc, copper, iron, manganese and magnesium were estimated from the inoculated soil by using Merwin and Peach (1951) method. For plant analysis, the whole plant was dried individually and powdered. 100 mg of plant powder samples was taken in 150 mL conical flask containing 10 mL nitric acid (HNO₃) and perchloric acid (HClO₄) in 9: 4 ratios. The flasks were placed on a hot plate and digested at 300°C until the entire plant material turned colorless. The complete extract was taken in 100 mL volumetric flask and the volume was made to 100 mL with distilled water. These samples were used for estimation of phosphorus potassium (Piper 1966), calcium, Zinc, magnesium, copper and iron by Jackson (1973) method. The total nitrogen content of soil and plant was analyzed by using Kjeldhal (1883) method.

Statistical Analysis

The statistical analysis of the study was done by using Agri statistical package (WASP 1.0), Goa.

RESULTS

Isolation of Actinomycetes from the AM Fungal Spores

Based on the morphology ten actinomycetes sp were isolated from the *glomus mosseae* spore crude extract. The molecular identity of the ten isolates was confirmed as (*Streptomyces fradiae*, *S. avermitilis*, *S. cinnamomensis*, *Leifsonia poea*, *S. canus*, *S. netropis*, *S. scabiei*, *S. albidoflavus*, *S. violaceus*, *S. gresius*) (Sukhada et al., 2013).

Antifungal Activity against *Fusarium solani*

In Dual culture assay, *Streptomyces cinnamomensis* showed highest growth inhibition on *Fusarium solani* followed by *Streptomyces avermitilis* and *Streptomyces netropis* Figure (2-a).

Isolation of Chitinase Gene from the Actinomycetes and Protein Bioassay on *Fusarium solani*

The amplified fragment of 1.15kb (Figure 1-a) from the g-DNA was further purified, confirmed by sequence analysis and also ligated into the PQE UA 30 expression vector. The restriction analysis (Figure 1-b) confirmed the positive clones for the expression studies. the expressed proteins from M15 E.coli cells was harvested and analyzed through poly acrylamide gel electrophoresis (Figure 1-c). The 31 KDa column purified proteins (Figure 1-d) were

quantified (320µg/ml) and used further for the bioassay. The 100µl of each of the purified proteins was inoculated individually in the 2mm dia wells scooped out in the well grown fungal plates and the plates were incubated at 30⁰ C. After 24 hours of incubation the zone of growth inhibition was found greater (Figure 2- b) in *S. cinnamonensis* (1.3±0.16 cm) inoculated plates followed by *S. avermitilis* (1.1±0.08 cm) and *S. netropis* (0.9±0.16 cm) Table 3.

Growth Enhancement Activity by Actinomycetes

All the Actinomycetes cultures inoculated to guava seedlings during growth period caused significant improvement in growth, the isolates showing varied differences in their effect (Table 4). Out of ten isolates the total plant dry matter was found more in *S. albidoflavus* inoculated plants (0.856±0.0 g) followed by *S. violarius* (0.437±0.0 g) and control plants (0.070±0.01 g). The maximum shoot length was observed in *S. albidoflavus* (0.437±0.0 g) followed by *S. violarius* (15.2±2.4 cm) inoculated plants. Increased root length was observed in *S. griseus* inoculated plants (49.3±1.24cm) followed by *S. cinnamonensis* (31.0±0.81 cm). Compared with control leaves the increased leaf area was observed in *S. canus* (2.6±0.16 cm²) followed by *S. violarius* (2.5±0.16 cm²) inoculated plants.

Analysis of Nutrient Content in the Inoculated Soil and Plant

The nutrient contents of the Actinomycetes treated plants and soils were greatly enhanced, the degree of enhancement showing variation among the isolates. The plant total N was found more in *S. canus* (1.93±0.10 %) inoculated plants followed by *S. avermitilis* (1.64±0.11 %), *S. cinnamonensis* (1.4±0.12 %) and *S. violarius* (1.36±0.08). The soil N was highest in *S. scabiei* (894.24±0.81 ppm) followed by *S. violarius* (719.28±1.64 ppm), *S. fradiae* (738.72±2.4 ppm) and *S. canus* (680.4±3.26 ppm). The P content of the plant was found more in *S. cinnamonensis* (0.57±0.01 %) inoculated plants followed by *S. avermitilis* (0.49±0.03 %) and *Leifsonia poea* (0.47±0.0 %). Soil P in the *S. cinnamonensis* inoculated plants was more (44.43±0.84 ppm) followed by *L. poea* (41.28±0.86 ppm) and *S. avermitilis* (34.92±0.82 ppm). The *S. fradiae* inoculated plants showed more K (6.15±0.12 %) content followed by *S. cinnamonensis* (5.75±0.28 %) and *S. avermitilis* (5.4±0.24 %). *S. cinnamonensis* inoculation showed more soil K content (1606.25±0.04 ppm) followed by *S. fradiae* (1475.0±4.08 ppm) and *S. violarius* (1387.5±1.02 ppm). Calcium content in the plant was found more in *S. avermitilis* (130.07±4.9 %) inoculated plants followed by *S. fradiae* (110.03±4.0%) and *S. cinnamonensis* (104.26±2.4 %). Highest soil-calcium content was observed in *S. fradiae* (2363.6±2.57 ppm) followed by *S. avermitilis* (1735.26±2.05 ppm) and *S. canus* (1709.3±3.26 ppm). Mg content of the *S. fradiae* inoculated plant was found more (2.3±0.2 %) followed by *S. violarius* (2.2±0.16 %) and *S. avermitilis* (2.17±0.05 %). Soil Mg content was observed high in *S. fradiae* (363.4±0.81 ppm) followed by *S. violarius* (366.16±1.32 ppm) and *S. griseus* (362.66±1.71 ppm). The Fe content of the *S. albidoflavus* inoculated plant was observed more (13913.25±2.44 ppm) followed by *S. canus* (10312.5±9.79 ppm) and *S. scabiei* (9887.0±1.63 ppm). Soil Fe content was more in *L. poea* treatment (846.66±1.68 ppm) followed by *S. violarius* (181.87±0.87 ppm) and *S. fradiae* (134.68±1.63 ppm). The plants inoculated with *S. albidoflavus* showed high Mn content (193.1±2.4 ppm) followed by *L. poea* (189.4±0.81 ppm) and *S. cinnamonensis* (171.4±2.44 ppm). The soil-Mn was high in *S. canus* (175.31±1.71 ppm) followed by *S. avermitilis* (162.81±1.63 ppm) and *L. poea* (157.81±0.81 ppm). Zinc content was high in *L. poea* (124.6±3.2 ppm) inoculated plants followed by *S. avermitilis* (120.8±1.71 ppm) and *S. violarius* (114.0±3.26 ppm). Soil Zn content was more in *S. avermitilis* (548.43±1.65 ppm) followed by *S. albidoflavus* (509.37±0.81 ppm) and *S. fradiae* (444.68±0.85 ppm). Cu was found more in plants treated with *S. albidoflavus* (517.5±1.63 ppm) followed by *S. violarius* (503.25±2.44 ppm) and *S. avermitilis* (403.75±2.44 ppm) treated plants. *S. canus* inoculation raised the Cu content (53.12±0.83 ppm) followed by *S. cinnamonensis* (52.82±0.83 ppm) and *S. scabiei* (52.81±0.82 ppm).

DISCUSSIONS

Actinomycetes are known for their plant growth promotion and effective antagonistic activity against wide range of plant pathogens. In the present study the ability of ten actinomycetes isolated from the AM fungal spore (*Glomus mosseae* collected from the guava rhizosphere) to promote growth, mobilize nutrition and exhibit antagonistic potential against *Fusarium solani* causing severe wilt disease in guava was studied. Chitinase genes were mined from the isolates and the chitinase protein expressed by the E.coli were purified and tested for inhibition of *F. solani*. Nutrients play a major role in plant growth and their defense against plant pathogens. Most of the soil nutrients are in the bound form and are not freely available to plants for absorption. To solubilize the soil bound nutrients the application of synthetic chemicals will cause soil infertility by deposition of toxic metal ions (Jeffries et al. 2003). But the application of soil microorganisms which can effectively solubilize the soil bound nutrients will facilitate the plant growth by enhanced absorption capacity (Glick, 2012). Many PGP bacteria are known for their ability to promote plant growth, solubilize and mobilize the soil bound nutrients in fruit and vegetable crops (Jilani et al. 2007; Vyas and Gulati, 2009). Among the bacteria's the wide group of actinomycetes are studied the maximum for their antagonistic potential (Aldequy et al. 1998; Oskay, 2009; Kaur et al. 2013) but the reports regarding nutrient mobilization by actinomycetes are considerably less (Mukesh Sharma, 2014). Franco-correa et al. 2010 reported the nutrient enhancement in *Glomus mosseae* and *Streptomyces* inoculated plants compared with combined inoculation the plants inoculated with individual *Streptomyces* isolates showed more N and P uptake (2.7 ppm and 0.33 ppm) in inoculated plants. Likewise in the present study, *S. avermitilis* inoculated guava plants had shown the highest N content (1.64 ± 0.11 %) followed by *S. cinnamomensis* (1.4 ± 0.12) inoculated plants. Whitelaw, (2000) reported the *Bacillus*, *pseudomonas*; *Rhizobium* and *Enterobacter* are the most important strains for the P solubilization. In the present study the P uptake was observed more in *S. griseus* (0.69 ± 0.03 %) and *S. cinnamomensis* (0.57 ± 0.01 %) inoculated plants. The P solubilizing isolates also have the ability to promote the plant growth by increasing the availability of the micro elements like iron, zinc (Ngoc et al., 2006). In the present study the enhanced Fe and Zn uptake was observed in *S. albidofalves* (13913.25 ± 2.44 ppm) and *Leifsonia poea* (124.6 ± 3.2 ppm) inoculated plants along with increased plant dry matter ($0.856 \pm 0.0g$). Khan (2005) reported increased uptake of Fe, Ca, Mg, Zn, K and P in the inoculated plants. In the present study the enhanced uptake of Ca, Fe and Zn was observed in guava plants with individual inoculation and the same was recorded in soil nutrient content compared to controls. The increased leaf area of the inoculated plants shows the enhanced uptake of Ca and Zn ions. Other than nutrient mobilization most of the plants growth promoting bacterial species act as biocontrol agent for many of the plant pathogens (Singh et al, 1996; Franskowski et al. 2001; Prapagdee et al., 2008; Sukhada et al. 2013) by means of production of hydrolytic enzymes and antibiotics (Mukherjee G and Sen SK, 2004; Luthara and Dubey, 2012). Kim et al. 2008 reported the Chitinolytic efficiency of plant growth promoting bacteria against the *phytophthora* blight in pepper plant. Singh et al. 1999 reported the bacterial Chitinolytic activity on *Fusarium* for the cucumber wilt. In the present study the antifungal activity of the actinomycetes was evaluated against *Fusarium solani* guava wilt disease. Out of ten isolates *S. cinnamomensis* showed high growth inhibition on *F. solani* followed by *S. avermitilis* and *S. netropis*. The same activity was further exhibited in chitinase gene mining in respective isolates. The expressed protein of the respective genes was tested on *F. solani*. The high growth inhibition was found in *S. cinnamomensis* (1.3 ± 0.16 cm) followed by *S. avermitilis* (1.1 ± 0.08 cm) and *S. netropis* (0.9 ± 0.16 cm). In the present study the ten isolates showed great variation in their ability to mobilize the solubilized nutrients from soil to plants and the background information about the plant and microbe interaction in soil is still unclear.

CONCLUSIONS

The present study demonstrates that Actinomycetes have a great potential to solubilize and mobilize nutrients when inoculated to soil. These studies were carried out under poly-house condition and further exploitation of these isolates on field for large scale application will clearly reduce the input of chemical fertilizers into the soil and will facilitate to develop broad spectrum biocontrol agents for sustainable agriculture.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Ragupathy, Principal scientist, Division of Soil Science, IIHR, for his valuable guidance during the nutrient analysis in soil and plant.

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APPENDICES

Figure 1: (a) Amplification of chitinase gene (1.1kb) from g-DNA. Lane 1-*Streptomyces netropis*, lane 2-*S. avermitilis* lane 3- *S. cinnamomensis*, lane 4- control Lane 5-1Kb DNA marker. (b) Restriction analysis for cloned product (1.1kb) from the vector system, Lane 1&5 – 1Kb DNA marker, Lane 2-4 clones of *S. netropis*, *S. avermitilis* and *S. cinnamomensis*. (c) poly acrelamide gel analysis for confirmation of expressed protein, Lane 1-3 unpurified protein samples (30 KDa) , lane 4- vector alone, Lane 5- pre-stained protein ladder ,Lane 6-induced and lane 7- un-induced samples. (d) Ni- NTA column purified protein samples (31 KDa), Lane 1- *S. netropis*, Lane 2- *S. avermitilis*, Lane 3- *S. cinnamomensis*, Lane 4- vector control and Lane 5- Protein marker.

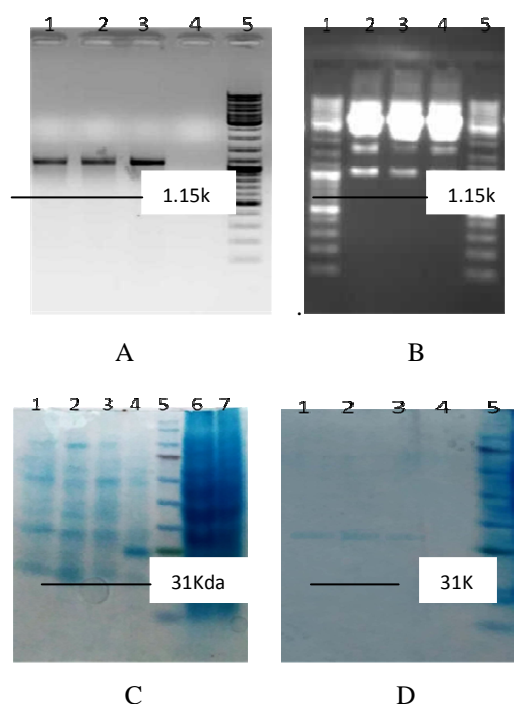


Figure 1

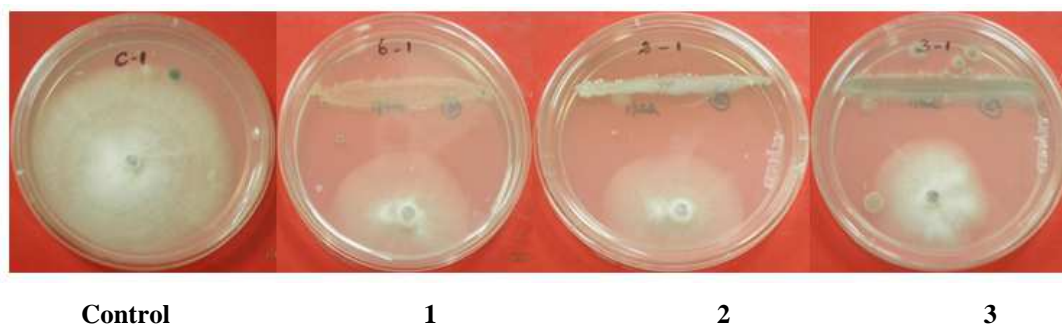


Figure 2(A): Antifungal Activity of Actinomycetes (*S. Netropis* (1), *S. avermitilis* (2), *S. cinnamonensis* (3)) Against *Fusarium solani* on Potato Dextrose Agar Plate

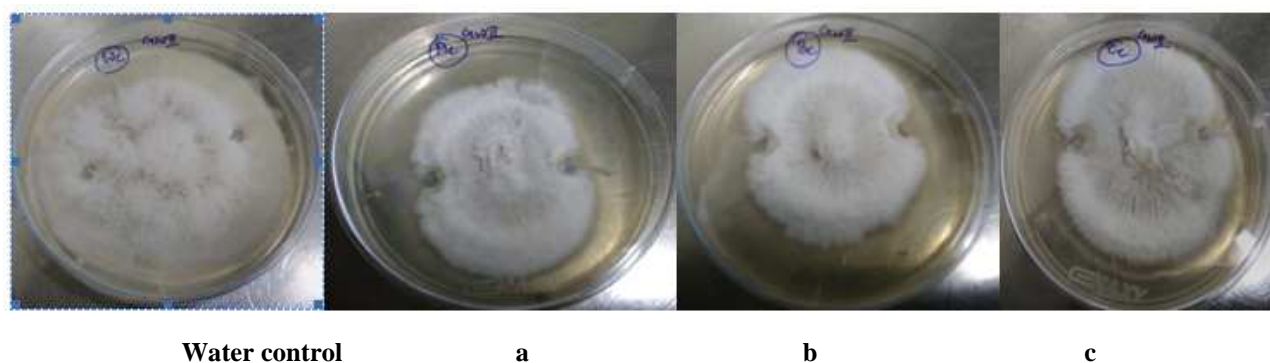


Figure 2: (B) Antifungal activity of purified chitinase protein from *S. netropis* (a), *S. avermitilis* (b), *S. cinnamonensis* (c) on *Fusarium solani*

Table 1: Nutrient Uptake by Plant Inoculated with Actinomycetes under Poly House Condition

Isolates	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
<i>Streptomyces fradiae</i>	1.3±0.08	0.44±0.03	6.15±0.12	110.03±4.0	2.3±0.2	2570.75±57.1	140.1±1.63	105.1±1.63	301.25±0.81
<i>S. avermitilis</i>	1.64±0.11	0.49±0.03	5.4±0.24	130.07±4.9	2.17±0.05	5819.0±15.5	163.3±1.41	120.8±1.71	403.75±2.44
<i>S. cinnamonensis</i>	1.4±0.12	0.57±0.01	5.75±0.28	104.26±2.4	2.07±0.46	7632.5±1.63	171.4±2.44	83.7±2.4	387.5±1.63
<i>Leifsonia poea</i>	1.2±0.08	0.47±0.0	2.0±0.48	99.54±1.63	1.72±0.08	9237.5±5.71	189.4±0.81	124.6±3.2	391.5±0.81
<i>S. canus</i>	1.93±0.10	0.20±0.01	2.5±0.40	112.04±1.63	1.95±0.08	10312.5±9.79	176.8±1.95	98.1±1.6	412.5±1.63
<i>S. netropis</i>	1.16±0.08	0.36±0.0	2.5±0.16	96.71±1.58	1.69±0.07	3330.25±24.4	170.4±1.79	83.8±2.4	382.3±1.63
<i>S. scabiei</i>	1.16±0.08	0.22±0.01	2.1±0.08	113.91±2.4	2.09±0.07	9887.0±1.63	190.1±0.81	91.3±0.81	452.5±1.63
<i>S. albidoflavus</i>	1.25±0.08	0.29±0.01	4.4±0.16	114.44±1.6	1.91±0.04	13913.25±2.44	193.1±2.4	114.0±3.26	517.5±1.63
<i>S. violaceus</i>	1.36±0.08	0.20±0.0	1.7±0.16	99.59±0.81	2.2±0.16	8693.75±2.44	145.9±4.08	90.2±1.71	503.25±2.44
<i>S. gresius</i>	1.17±0.05	0.69±0.03	2.2±0.16	106.94±1.6	1.78±0.14	8231.25±0.81	170.4±1.63	96.0	370.0±1.63
Control	0.95±1.2	0.16±0.0	1.3±0.24	90.45±1.67	1.25±0.12	1580.75±65.3	82.1	73.2	207.5±1.63
SEM	0.254	0.165	1.70	10.30	0.285	3556.42	30.25	15.56	82.67
CD at 5% P(0.05)	0.204	0.041	0.509	5.215	0.380	57.723	4.469	4.458	3.537

Table 2: Nutrient Content of the Soil Inoculated with Actinomycetes under Poly House Condition

Isolates	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
<i>Streptomyces fradiae</i>	738.72±2.4	30.52±0.83	1475.0±4.08	2363.6±2.57	363.4±0.81	134.68±1.63	86.81±0.82	444.68±0.85	47.5±0.81
<i>S. avermitilis</i>	670.68±4.0	34.92±0.82	1206.25±1.67	1735.26±2.05	358.1±1.63	111.25±0.85	162.81±1.63	548.43±1.65	40.0±0.81
<i>S. cinnamonensis</i>	651.24±0.03	44.43±0.84	1606.25±0.04	1210.0±8.16	308.03±1.01	123.75±1.67	129.06±1.63	373.12±0.83	52.82±0.83
<i>Leifsonia poea</i>	625.60±3.40	41.28±0.86	968.75±2.55	1645.9±1.63	364.6±1.63	846.66±1.68	157.81±0.81	340.93±0.84	33.75±2.49
<i>S. canus</i>	680.4±3.26	27.11±1.63	887.5±1.63	1709.3±3.26	355.3±1.63	125.6±1.63	175.31±1.71	295.0±0.81	53.12±0.83
<i>S. netropis</i>	612.36±1.68	23.38±0.88	1018.75±1.67	1120.3±1.63	348.4±1.71	132.8±1.63	146.93±1.65	315.7±0.81	43.56±2.44
<i>S. scabiei</i>	894.24±0.81	29.39±0.31	1231.25±0.81	1249.3±0.81	357.1±0.81	114.37±1.69	91.87±0.81	427.5±1.63	52.81±0.82
<i>S. albidoflavus</i>	665.82±1.64	30.60±0.82	1325.0±4.0	1324.4±1.67	354.3±0.89	122.8±1.63	79.2±0.73	509.37±0.81	41.25±0.85
<i>S. violarius</i>	719.28±1.64	23.90±0.84	1387.5±1.02	1620.9±1.63	366.16±1.32	181.87±0.87	73.75±0.93	307.5±0.81	45.31±0.82
<i>S. gresius</i>	636.66±0.80	22.31±1.64	1275.0±1.63	1695.6±1.63	362.66±1.71	111.25±0.93	83.2±0.89	444.37±0.87	52.18±1.63
Control	534.6±0.81	17.87±0.45	800.0±1.63	1050.80±1.67	302.8±1.61	109.37±1.69	60.5±1.63	230.0±1.63	30.37±0.81
SEM	86.51	7.719	241.59	361.59	21.23	207.85	39.58	93.46	7.55
CD at 5% P(0.05)	4.657	2.044	4.644	6.676	2.917	3.088	2.648	2.314	2.821

Table 3: Growth Inhibition of *Fusarium solani* by Purified Chitinase Proteins of Actinomycetes

Gene Isolated from	Zone of Inhibition in (cm)
<i>Streptomyces cinnamonensis</i>	1.3±0.16
<i>Streptomyces avermitilis</i>	1.1±0.08
<i>Streptomyces netropis</i>	0.9±0.16
Control	0.0
SEM	0.496
CD at 5% P(0.05)	0.282

**Table 4: Growth Promotion Activity of Actinomycetes in 5 Month Old Guava CV
Arka Kiran Seedlings under Poly House Condition**

Isolates	Plant Shoot Height(cm)	Plant Root Height(cm)	Leaf Width (cm ²)	Shoot Dry Weight(g)	Root dry Weight(g)	Total Biomass(g)
<i>Streptomyces fradiae</i>	10.3±0.65	28.0±1.63	2.1±0.08	0.127±0.0	0.069	0.196±0.0
<i>S. avermitilis</i>	10.0±0.43	27.0±0.81	1.8±0.16	0.122±0.0	0.082±0.0	0.204±0.0
<i>S. cinnamonensis</i>	8.0±0.81	31.0±0.81	1.4±0.16	0.283±0.0	0.080±0.0	0.360±0.0
<i>Leifsonia poea</i>	10.7±0.57	29.5±0.81	1.5±0.20	0.166±0.0	0.092±0.0	0.258±0.0
<i>S. canus</i>	14.5±2.04	26.0±1.63	2.6±0.16	0.283±0.0	0.131±0.0	0.414±0.0
<i>S. netropis</i>	10.8±0.65	25.0±0.81	1.2±0.16	0.110±0.0	0.040±0.0	0.150±0.0
<i>S. scabiei</i>	11.2±0.81	29.0±1.63	1.7±0.16	0.147±0.0	0.040±0.0	0.187±0.00
<i>S. albidoflavus</i>	15.5±1.63	27.0±0.81	2.2±0.16	0.522±0.0	0.334±0.0	0.856±0.0
<i>S. violarius</i>	15.2±2.4	30.0±0.81	2.5±0.16	0.301±0.0	0.136±0.0	0.437±0.0
<i>S. gresius</i>	9.0±0.81	49.3±1.24	1.4±0.16	0.172±0.0	0.068±0.0	0.240±0.0
Control	6.0±0.81	20.0±1.88	1.0±0.08	0.030±0.0	0.040±0.0	0.070±0.01
SEM	2.857	6.951	0.503	0.127	0.081	0.203
CD at 5% P(0.05)	2.605	2.519	0.324	0.011	0.011	0.014